Datasheet for ABIN649085

hCG ELISA Kit

Go to Product page

Overview

Quantity:	96 tests
Target:	hCG
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	Immunoenzymometric assay (TYPE 3): The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-hCG antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies without competition or steric hindrance to form a soluble sandwich complex. After equilibrium is attained, the antibodybound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibodybound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.
Analytical Method:	Quantitative
Detection Method:	Colorimetric

Product Details

Components:	A. hCG Calibrators (1 ml/vial). Six vials of references for hCG Antigen at levels of 0 (A), 5 (B), 25 (C), 50 (D), 100 (E) and 250 (F) mIU/ml. Store at 2-8°C. A preservative has been added. Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 3rd IS (75/537). B. hCG Enzyme Reagent (13 ml/vial). One vial containing enzyme labeled affinity purified antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C. C. Streptavidin Coated Plate (96 wells). One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C. D. Wash Solution Concentrate (20 ml). One vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-30°C. E. Substrate A (7ml/vial). One bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. F. Substrate B (7ml/vial). One bottle containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C. G. Stop Solution (8ml/vial). One bottle containing a strong acid (1N HCl). Store at 2-30°C. H. Product Instructions: Note 1: Do not use reagents beyond the kit expiration date. Note 2: Opened reagents are stable for 60 days when stored at 2-8°C. Note 3: Above reagents are for a single 96-well microplate.
Material not included:	1. Pipette (s) capable of delivering 25 & 50 volumes with a precision of better than 1. 5%. 2. Dispenser(s) for repetitive deliveries of 0. 100ml and 0. 350ml volumes with a precision of better than 1. 5%. 3. Microplate washers or a squeeze bottle (optional). 4. Microplate Reader with 450nm and 620nm wavelength absorbance capability. 5. Absorbent Paper for blotting the microplate wells. 6. Plastic wrap or microplate cover for incubation steps. 7. Vacuum aspirator (optional) for wash steps. 8. Timer. 9. Quality control materials.

Target Details

Target:	hCG
Abstract:	hCG Products
Target Type:	Hormone
Background:	Summary and Explanation of the test: Human chorionic gonadotropin (hCG) concentration increases dramatically in blood and urine during normal pregnancy. hCG is secreted by placental tissue, beginning with the primitive trophoblast, almost from the time of implantation, and serves to support the corpus luteum during the early weeks of pregnancy. hCG or hCG similar glycoproteins can also be produced by a wide variety of trophoblastic and nontrophoblastic tumors. The measurement of hCG, by assay systems with suitable sensitivity and specificity has proven great value in the detection of pregnancy and the diagnosis of early pregnancy disorders. According to the literature, hCG is detectable as early as 10 days after

Target Details

ovulation, reaching 100 mIU/ml by the first missed period. At the time for the next ovulation, the hCG level is 200 mIU/ml (approximately 28 days after conception) (1). A peak of 50,000 or even 100,000 mIU/ml is attained by the third month, then a gradual decline is observed (2, 3). In this method, hCG calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of hCG) are added and the reactants mixed. Reaction between the various hCG antibodies and native hCG forms a sandwich complex that binds with the streptavidin coated to the well. After the completion of the required incubation period, the enzyme-chorionic gonadotropin antibody bound conjugate is separated from the unbound enzyme-chorionic gonadotropin conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color. The employment of several serum references of known chorionic gonadotropin levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with chorionic gonadotropin concentration. Intended Use: The Quantitative Determination of Chorionic Gonadotropin (hCG) Concentration in Human Serum by a Microplate Immunoenzymometric assay. Q. C. Parameters: In order for the assay results to be considered valid the following criteria should be met: 1. The absorbance (OD) of calibrator F should be greater than 1.3. 2. Four out of six quality control pools should be within the established ranges.

Application Details

Application Notes:	Precautions: All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, Biosafety in Microbiological and Biomedical Laboratories, 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.
Plate:	Pre-coated
Reagent Preparation:	1. Wash Buffer: Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27 °C for up to 60 days. 2. Working Substrate Solution: Pour the contents of the amber vial labeled Solution A into the clear vial labeled Solution B. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8 °C. Note: Do not use the working substrate if it looks blue.

Application Details

Sample Collection:	The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells. Samples may be refrigerated at 2-8°C for a maximum period of five days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20 °C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0. 05 ml of the specimen is required.
Calculation of Results:	A dose response curve is used to ascertain the concentration of Human chorionic gonadotropin (hCG) in unknown specimens. 1. Record the absorbance obtained from the printout of the microplate reader. 2. Plot the absorbance for each duplicate serum reference versus the corresponding hCG concentration in mIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting). 3. Draw the best-fit curve through the plotted points. 4. To determine the concentration of hCG for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). Note: Computer data reduction software designed for IEMA/ Elisa assays may also be used for the data reduction.
Restrictions:	For Research Use only

Handling

Handling Advice:	Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C). 1. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C. 2. Pipette 0. 025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well. Add 0. 100 ml (100µl) of hCG-Enzyme Reagent to all wells. 4. Swirl the microplate gently for 20-30 seconds to mix and cover. 5. Incubate 60 minutes at room temperature. 6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper. 7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two additional times for a total of three washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and Repeat two additional times. 8. Add 0. 100 ml (100µl) of working substrate solution to all

Handling

	wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION. 9. Incubate at room temperature for 15 minutes. 10. Add 0. 050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds). Always add reagents in the same order to minimize reaction time differences between wells. 11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within 30 minutes of adding the stop solution.
Storage:	4 °C/-20 °C